REMARKS

I. Status of Claims:

Upon entry of the present amendment, claims 1, 2, 4, 9, 12, 15-21, and 23-36 will be pending and under examination in this application. Claims 1, 9, and 30 are presently amended; claim 22 is canceled herein without prejudice; and claims 31-36 are newly added. Support for the amended and new claims can be found, for example, at page 4, line 35 to page 5, line 7; page 6, lines 24-30; page 8, lines 2-5; and Example 2 at page 19 of the application as filed. No new matter is added.

II. Rejections Under 35 U.S.C. § 112, First Paragraph:

Claims 1, 4, 9, 12, 15-22, and 28-30 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not being enabled for methods wherein the lesional tissue is an arteriosclerotic lesion. *See*, Office Action at pages 3-4. According to the Office Action at page 3, the specification is enabling for methods wherein the lesional tissue is a cancer tissue, an inflammatory disease lesion, a lesion generated by an infectious pathogen, an autoimmune disease lesion, or an artificially prepared lesion, but not for methods wherein the lesional tissue is an arteriosclerotic lesion. Applicants have canceled claim 22, thereby rendering the rejection moot as to that claim. The remaining claims under rejection are now limited to a scope that the Office Action acknowledges is sufficiently enabled by the specification.

In view of the present amendment to the claims, Applicants respectfully submit that the grounds for this rejection under 35 U.S.C. § 112, first paragraph, have been overcome.

III. Rejections Under 35 U.S.C. § 103(a):

(a) Claims 1, 2, 4, 15-21, 27, and 30 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Obiakor *et al.* (*Analytical Biochemistry*, **306**:55-62 (2002)) in view of Kotlan *et al.* (*Immunology Letters*, **65**:143-151 (1999)). *See*, Office Action at pages 5-7. Applicants traverse.

Obiakor is directed to studying rearrangement of VDJ regions by gene conversion and hypermutation by recovery of single cells from normal rabbit appendix or spleen or normal human appendix, and analysis of their DNA by PCR and sequencing, without a cloning step. There is no teaching or suggestion in Obiakor of recovering single cells from cancerous tissue or of isolating mRNA, let alone of cloning and expression of antibody-encoding sequences.

Kotlan is directed to studying the immunoglobulin repertoire of B-lymphocytes infiltrating a high grade breast medullary carcinoma. Kotlan does this by isolating RNA from a suspension of a *plurality* of TIL-B cells, preparing cDNA, and performing PCR using primers for rearranged immunoglobulin variable regions. There is no teaching or suggestion in Kotlan of recovering single cells for cloning and expression of antibody encoding sequences.

The Office Action's rationale for combining Kotlan with Obiakor is that Kotlan allegedly teaches that "B cells are present in lesional tissues such as breast cancers and nucleotides encoding antibodies may be isolated from these B cells." *See*, Office Action at page 6, first full paragraph. The Office Action also alleges that "Obiakor teaches that when DNA is derived from multiple germinal center B cells instead of single cells a PCR artifact hybrid gene is formed (see page 55, right column). Therefore, Obiakor provides a reason for isolating a single B cell as a source of immunoglobulin DNA." *See*, Office Action at paragraph bridging pages 6-7.

Applicants submit that the combined references do not render Applicants' invention obvious for at least the following reasons.

First, there is no reasonable expectation of success that the LCM technique (what Applicants refer to as LMD) employed by Obiakor in normal tissues would work in lesional tissues. Lesional tissues are generally more disorganized and thus presumably difficult to manipulate than normal tissues. *See e.g.,* Ingber et al., *Proc. Natl. Acad. Sci. USA*, **78(6)**:3901-3905 (1981); and Grossman, *Haematology and Blood Transfusion*, 31: 289-298 (1987), which are attached to this Amendment as **Attachments A and B**. It is unpredictable from the teachings of the combined references whether the LMD technique would be effective in permitting isolation of single TIL-B cells from lesional tissues. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.,* 82 USPQ2d 1385, 1396 (2007).

Second, the claims as presently amended require, in relevant part, that mRNA from the isolated B cell be purified after addition of (i) carrier cells that do not express any antibody genes, or (ii) carrier RNAs that are not antibody-encoding transcripts. Applicants note that Obiakor and Kotlan, either alone or in combination, nowhere provide a reason to add carrier RNA or carrier cells that lack any antibody genes during the step of purifying mRNA from the isolated B cell. Obiakor is concerned with genomic DNA (not mRNA) extraction for PCR of the VDJ region to study gene conversion, so would have had no need to purify RNA, much less to add carrier RNA or carrier cells prior to purifying RNA. Kotlan describes preparing RNA from suspensions of a plurality of cells, as opposed to single cells, so for different reasons also had no need to add carrier RNA or carrier cells. Carrier RNA or carrier cells are utilized when the amount of RNA of interest is so minute that manipulation of it is difficult or impossible. This was apparently not the case in Kotlan. Thus, neither Obiakor nor Kotlan provides a reason to carry out the method as claimed. Further, neither provides an expectation that one would be able to purify RNA from a single cell, with or without the addition of carrier RNA or carrier cells.

For at least the foregoing reasons Applicants submit that rejected claims 1, 2, 4, 15-21, 27, and 30 and new claims 31-36 are not obvious over Obiakor and Kotlan and thus request that this rejection be reconsidered and withdrawn.

(b) Claims 9, 12, 28 and 29 remain rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Obiakor in view of Zhang *et al.* (*Cancer Research*, **55**:3584-3591 (1995)). *See*, Office Action at pages 7-8.

Obiakor is discussed in rejection (a) above.

Zhang teaches the use of Epstein Bar Virus (EBV) transformation of TIL-B cells and cloning by limiting dilution to identify antitumor antibodies.

Applicants submit that the combined teachings of these references do not render obvious Applicants' claimed invention for at least the reasons set out below.

First, as pointed out above, there is no reasonable expectation of success that the LCM technique (what Applicants refer to as LMD) employed by Obiakor in normal tissues would work in lesional tissues. As explained above, lesional tissues are generally more disorganized and difficult to manipulate than normal tissues. It is unpredictable from the teachings of the

combined references whether the LMD technique would be effective in permitting isolation of single TIL-B cells from lesional tissues.

Second, modifying Zhang as suggested by the Office Action would make Zhang's invention unsatisfactory for its intended purpose. Zhang is directed at obtaining tumor-specific antibodies. Zhang accomplishes this goal by transforming TIL-B cells with EBV and determining which of the B cell transformants express antibodies that are reactive against autologous and allogeneic tumors (see, "Detection of Antitumor Antibodies" and "Cloning EBV Transformed B Cells by Limiting Dilution" on page 3585, right column). The tumor-specific B cell lines are then cloned by limiting dilution. If Zhang's EBV transformation and limiting dilution of B cells is replaced with the LMD technique of Obiakor, Zhang would not be able to test *a priori* whether an antibody produced by a B cell is tumor specific because there presumably would not be enough antibody produced by a single cell (as opposed to a population of cloned cells) to test against the panel of tumors to identify tumor-specific antibody-producing B cells. The antibody-testing step is integral to Zhang's teachings. If a proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900 (Fed. Cir. 1984).

Third, the claims as presently amended require, in relevant part, that mRNA from the isolated B cell be purified after addition of (i) carrier cells that do not express any antibody genes, or (ii) carrier RNAs that are not antibody-encoding transcripts. Applicants note that Obiakor and Zhang, either alone or in combination, nowhere provide a reason to add carrier RNA or carrier cells that lack any antibody genes during the step of purifying mRNA from the isolated B cell. Obiakor is concerned with genomic DNA (not mRNA) extraction for PCR of the VDJ region to study gene conversion, so would have had no need to purify RNA, much less to add carrier RNA or carrier cells prior to purifying RNA. Zhang describes preparing RNA suspensions of a plurality (10^6 cells/ml) of EBV transformed B cells, as opposed to single cells, so had no need to add carrier RNA or carrier cells. Carrier RNA or carrier cells are utilized when the amount of RNA of interest is so minute that manipulation of it is difficult or impossible. This was apparently not the case in Zhang. Thus, neither Obiakor nor Zhang provides a reason to carry out the method as claimed. Further, neither provides an expectation

that one would be able to purify RNA from a single cell, with or without the addition of carrier RNA or carrier cells.

Applicants submit that rejected claims 9, 12, 28, and 29 and new claims 31-36 are not obvious over Obiakor and Zhang and thus request that this rejection be reconsidered and withdrawn.

(c) Claims 1, 2, 4, 15-21, 23-26, and 30 remain rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Obiakor in view of Mallison et al. (*Infection and Immunity*, **59(1)**:4019-4025 (1991)). *See*, Office Action at page 8.

Obiakor is discussed in rejection (a) above.

Mallison is directed to determining the effects of chronic inflammation, nonspecific activator, and specific antigen alone and in combination on the local accumulation of antibody forming cells (AFCs). Mallison teaches that the combination of chronic inflammation, nonspecific activator, and specific antigen is the most potent recruiter of AFCs.

Applicants submit that the combined teachings of these references do not render obvious Applicants' claimed invention because there is no reasonable expectation of success that the LCM technique (what Applicants refer to as LMD) employed by Obiakor in normal tissues would work in lesional tissues. As discussed above, lesional tissues are generally more disorganized and difficult to manipulate than normal tissues. It is unpredictable from the teachings of the combined references whether the LMD technique would be effective in permitting isolation of single infiltrating B cells from lesional tissues.

Also, the claims as presently amended require, in relevant part, that mRNA from the isolated B cell be purified after addition of (i) carrier cells that do not express any antibody genes, or (ii) carrier RNAs that are not antibody-encoding transcripts. Applicants note that Obiakor and Mallison, either alone or in combination, nowhere provide a reason to add carrier RNA or carrier cells that lack any antibody genes during the step of purifying mRNA from the isolated B cell. In fact, neither Obiakor nor Mallison discloses anything about preparing RNA. Obiakor is concerned with genomic DNA (not mRNA) extraction for PCR of the VDJ region to study gene conversion, and Mallison merely counted cells in tissue sections.

For at least the foregoing reasons, Applicants submit that rejected claims 1, 2, 4, 15-21, 23-26, and 30 and new claims 31-36 are not obvious over Obiakor and Mallison and thus request that this rejection be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that all outstanding rejections are overcome by the present amendment. Accordingly, Applicants request the timely issuance of a Notice of Allowability. If the Examiner wishes to discuss this case, she is invited to call the undersigned at the telephone number provided below.

Other than the RCE filing fee, no fees are believed to be due. Please apply any charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 14875-0144US1.

Respectfully submitted,

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